Support for new claim 61 may be found, for example, page 31, last paragraph.

Relying on 35 U.S.C. §103, the Examiner rejected claims 39 to 47 and 50, 51 and 55 alleging they are obvious over Sanders et al. in view of Alberts et al. or Watson et al. Relying on the same statutory provision the Examiner also rejected claims 52 to 54 and 56 to 60 alleging they are obvious over Sanders et al. in view of Alberts et al. or Watson et al. in further view of Axel et al. Applicants respectfully traverse these rejections.

First, the Examiner does not state why a skilled artisan should seek to clone, sequence and express a gene involved in any metabolically important pathway. For even if it is accepted *arguendo* that there is a general desire among skilled artisans to clone genes, this does not make the cloning of a <u>particular gene</u> obvious. If the argument presented by the Examiner is accepted, then it becomes "obvious" to clone every gene known. Clearly this is not so.

Applicants submit that the fact that a gene could be cloned, in and of itself, does not render obvious the cloning of a particular gene. The Examiner is surely aware that mammalian species are estimated to have perhaps 100,000 genes. Applicants submit that the cloning of a particular <u>one</u> of these 100,000 genes is not rendered obvious but rather is a skill of the inventive artisan. The Examiner apparently fails to realize that a substantial part of the inventive activity involved in cloning and

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expressing a particular gene is the selection of the gene for cloning in the first place.

At the priority date of the present application there would have been little motivation to clone the GS gene, since the GS gene is endogenous to the majority of cell lines and, absent the disclosure of the present application, the skilled person would not, at the time, have been aware of any important uses of the cloned gene.

However, by demonstrating that a cloned, exogenous GS gene is amplifiable in a mammalian cell, even in the presence of the endogenous gene, the present Applicants have provided for the first time enormous incentive to clone the GS gene. This is because, before the present invention was made, commonly-used amplifiable genes were only known to work in cells which contained no endogenous equivalent. Therefor, for the first time, the present invention allows for a selectable gene system which may be used in the majority (if not all) cell lines, without the need for recourse to specialized, deficient cell lines which lack a particular endogenous gene.

It is further submitted that at the time of the present invention the ordinary artisan would have had little interest in cloning the GS gene. All that the skilled person had at his disposal before the priority date of the present application is the general techniques for cloning together with an apparently speculative disclosure (Sanders et al.) of a DNA fragment which may or may not have included part of the coding sequence for GS.

Applicants direct the Examiner's attention to the fact that there is no evidence that Sanders et al. cloned a DNA which actually encodes GS, save that the cloned DNA hybridizes to a mRNA which translates to a polypeptide which runs at a size of 42 kD in SDS-PAGE. There is absolutely no evidence in the Sanders et al. publication that this cloned DNA actually encodes GS. That publication does not provide data concerning the functional activity of the polypeptide produced by *in vitro* translation of the hybrid-selected mRNA. Neither does that publication provide immunoreactivity data with anti-GS antibody to lead the ordinary artisan to conclude that the protein made contains GS epitopes. It is entirely possible that the DNA of Sanders et al. encodes a different 42 kD protein based upon the data disclosed therein.

Of interest in this regard is that following statement of Sanders et al. on page 68, right column:

> "We have not yet determined the relationship between the multiple abundant mRNA species detected in the Northern blot of KG1MSC4-M poly(A) mRNA using pGS-1 as a probe (Figure 2b, tracks 2 and 3), and the production of functional GS protein."

This statement directly implies that there was the distinct possibility that the hybrid selected mRNA translated *in vitro* in Sanders et al. did not encode a functional GS protein. It is thus respectfully submitted that despite claims to the contrary in the Sander's et al. publication, there is no firm evidence disclosed in that publication to provide *prima facie* support for the conclusion stated therein that "at least part" of the GS gene was cloned.

The Examiner contends that the skilled artisan would have been induced to complete the studies begun by Sanders et al. It is respectfully submitted that this would not have been the case. The skilled artisan would have been skeptical of the results of Sanders et al. and would certainly have not been able to derive any advantage from them, since the quest to clone a GS gene would essentially have to be started from scratch, as there was no evidence that the DNA fragment of Sanders et al. in reality contained <u>any part</u> of the GS gene. Therefore, the disclosure of Sanders et al. is submitted not to assist the skilled person in any way in the cloning of the GS gene, nor does it provide any significant motivation for him to do so.

In addition to the above, although the endogenous, chromosomal GS gene can be amplified, the Sanders et al. publication states that 50 kb is the "minimum size estimate of the common amplified repeat unit" and that such a minimum unit "may or may not reflect the size of the minimum unit which the cell can amplify" (page 69). Thus, there clearly would have been doubt that the recombinant vector of the present invention would contain sufficient information to be amplified in a cell line. Sanders et al. do not show that the "at least part" of GS gene alleged to be contained in plasmid pGS-1 is amplifiable or, for that matter, provides any resistance to Msx. While this is pertinent to all claims, the Examiner's attention is respectfully directed to new claim 61 which features that the recombinant DNA be "amplifiable."

The further rejection advanced by the Examiner in view of Axel et al. appears to be somewhat off the point. Before the disclosure of the present application, it was not known that a cloned GS gene inserted into a cell as part of a vector could be amplified even in the presence of an endogenous GS gene in the genome of the cell.

Previous co-amplification systems, such as the DHFR system, require that the cell line used be deficient for the amplifiable gene. Thus, DHFR genes are only amplified in cells deficient for DHFR. In contradistinction, GS is amplified even in cell which possesses an endogenous GS gene. This is what is meant when it is stated that Axel et al. only disclose the use of exogenous genes as selectable markers. In the disclosure of Axel at al, it is essential that the gene to be amplified is not endogenous to the cell. This is what is reflected in the passage from Axel et al. quoted in the June 11, 1993 Official Action.

> "Selectable phenotype is a phenotype which confers upon an organism ability to exist under conditions which kill off all organisms not possessing the phenotype." (emphasis added)

Selection with the GS gene, contrary to the statement made by the Examiner, does not fall into this category. In fact, the GS gene has been found to be a selectable and amplifiable marker even in cell lines which, because they possess an endogenous GS gene, survive perfectly well under conditions of glutamine starvation. This is in clear contrast to the situation in which DHFR is used, wherein the absence of an endogenous DHFR gene is essential for the use of the DHFR gene as an amplifiable marker.

The Examiner further states that the Applicants have not disclosed any specific reasons why the GS gene would not function in the method disclosed by Axel et al. Applicants respectfully submit that this is hardly the point. What is important is that neither Axel et al. nor any other publication of record discloses that the GS gene would function in the method disclosed by Axel et al. The Examiner fills this gap in the rejection not by evidence of record, but apparently through use of the teachings of Applicants' specification. This is submitted to be improper hindsight.

Moreover, there is also no disclosure in the cited publications that the GS gene could function in a method quite different to that of Axel et al., and thereby providing a considerable advantage and significant advance in the art compared to the methods and selectable phenotype markers disclosed in Axel et al.

Claims 39, 40, 57 and 59 stand rejected because the Examiner contends that the disclosure of the present application is enabling only in respect to the hamster GS gene and CHO cells. The Examiner apparently feels that in order to isolate such genes at the time of the present invention one would have to develop cell lines from, among others, snow leopard. However, the most likely reason snow leopard cell lines do not exist is that there is presently no reason to make them, not that they cannot be made. If any cell lines were ever made from snow leopards, it is submitted to be a sound prediction that the GS gene could be amplified therein.

Further, cell lines are not necessary, as the DNA to be cloned could be isolated from any source, e.g. tissue as is known in the art. Thus, it is a feature of the present invention that it may be operated in all cell lines, not just CHO cells.

Relying on 35 U.S.C. §112, second paragraph, the Examiner maintains his rejection of claim 44, alleging the phrase "high stringency conditions" is indefinite. The Examiner apparently believes that the skilled artisan is unable to determine what is meant by this nearly universal term of art. While "high stringency conditions" may involve different salt concentrations, temperatures, etc., the claim terminology is well known not to reflect the individual concentrations, temperatures, etc., but rather the result of a given combination of these adjustable parameters, under which a DNA fragment binds specifically to a homologous sequence and does not bind to a substantially non-homologous sequence.

For the Examiner's convenience, submitted herewith are pages from Maniatis et al. relating the stringency of hybridization conditions. This publication was generally accepted as a textbook of the general methodology available to the person in the art at the time of the present invention. Maniatis et al. explains the stringency of DNA and RNA hybridization reactions in terms of the duplex melting temperature, Tm. See, for example, guideline 6 on page 325. Furthermore, Maniatis et al. set out how to determine empirically the relationship of Tm to temperature, salt concentration and solvent conditions (page 388, paragraph 11,

general "Note" and "a", "b" and "c", page 389 "Note b").

It is respectfully submitted that at the time the present invention was made, the skilled person in the art would have been aware of the meaning of "high stringency" and able to apply the well-known teaching of Maniatis to determine suitable conditions.

The Examiner also states that the "metes and bounds" of claim 44 cannot be determined for the phrase "a part thereof from a different species," since the "claim can potentially read on anything from the entire sequence of the GS gene to a single nucleotide of said gene." Applicants respectfully submit that in viewing the claim as a whole, a single nucleotide would not be said to hybridize under stringent conditions as this terminology is understood in the art.

For the reasons given, Applicants respectfully submit that the rejections should be withdrawn and that their application is now in condition for allowance. Applicants request notice of such allowability in the next communication from the Examiner.

The Examiner is invited to call the undersigned attorney should any minor matter remain.

Respectfully submitted,

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